

**Amendments to the Specification:**

Please replace paragraph [0007] with the following amended paragraph:

[0007] Thus, the present invention provides a method for designing primer pairs for amplifying a target sequence, comprising the steps of: choosing a reference sequence; removing at least selected repeat regions in the reference sequence to yield removed and unremoved reference sequence; selecting primer sequences from the unremoved reference sequence according to two or more parameters including primer length and primer melting temperature to yield a set of primers; evaluating the set of primers for extent of coverage and overlap of the reference sequence; and selecting a subset of primer pairs having reduced overlap from the set of primers.

Please replace paragraph [0027] with the following amended paragraph:

[0027] The third step 300 may be performed by hand or by a computer software program. For example, commercially available software such as Primer 3 ([www-genome.wi.mit.edu/cgi-bin/primer/primer3](http://www-genome.wi.mit.edu/cgi-bin/primer/primer3), incorporated herein by reference), xprimer ([alces.med.umn.edu](http://alces.med.umn.edu), Virtual Genome Center, incorporated herein by reference), Oligo (Molecular Biology Insights, Inc., Cascade, CO, incorporated herein by reference) or PrimerSelect (DNAStar, Inc., Madison, WI, incorporated herein by reference) may be employed. Those with skill in the art may be familiar with other programs that are available for primer selection or can develop such a program. In one embodiment, a software program is used that allows one to dictate various primer parameters such as primer melting temperature, primer length, stringency of hybridization, existence of duplexes, specificity of hybridization, existence of a GC clamp, existence of hairpins, existence of sequence repeats, the dissociation minimum for a 3' dimer, the dissociation minimum for the 3' terminal stability range, the dissociation minimum for a minimum acceptable loop, percent maximum homology, percent consensus homology, the maximum number of acceptable sequence repeats, frequency threshold, or the maximum length of acceptable dimers and the like. Also, in choosing primers for the third step, the length of a first primer of a primer pair may be fixed at a specific length, and the length of a second primer of the primer pair may be adjusted so

that the melting temperature of the second primer pair is substantially the same as the melting temperature of the first primer.

Please replace paragraph [0072] with the following amended paragraph:

[0072] In the present embodiment, the primer pair set output obtained from Oligo 6.52 was, in the fourth step of primer selection, subjected to Dijkstra's algorithm (again, see *Introduction to Algorithms*, Cormen, Rivest and Leiserson (1990); ISBN 0262031418)). The goal of this step being to find a best subset of primer pairs to amplify the target sequence out of all possible sets of primer pairs generated by Oligo 6.52. Dijkstra's algorithm solves the single-source shortest path problem on a weighted, directed graph. In the embodiment of this algorithm used in applications of this invention, each primer pair was considered a "vertex" with an "edge" defined for each pair of vertices. An associated "cost" was assigned to each edge where the cost reflected the amount of: 1) the overlap of vertices (cost = the length of the overlap); 2) the gap between two primer pairs (cost = 10x the length of the gap); and 3) a fixed value for having to add another vertex to the set (which increased the number of primers that must be used) (cost for additional primer pair = 4000). In one application of the present invention, the path with the lowest cost was selected, where total cost equals the sum of the costs of edges in the path. For example, assume three exemplary primer pairs:

	<u>5' position of the forward primer</u>	<u>5' position of the reverse primer</u>
Primer 1:	1000	2000
Primer 2:	1800	3000
Primer 3:	2100	4000

The "edges" are defined as being between Primer 1 and Primer 2, Primer 1 and Primer 3, and Primer 2 and Primer 3. The cost associated with the edge Primer1/Primer2 is  $200 + 10 ([10])0 + 4000 = 4200$  (reflecting the 200 base overlap between the amplicons). The cost associated with edge Primer1/Primer3 is  $0 + 10 (100) + 4000 = 5000$  (reflecting the 100 base pair gap between Primer 1 and Primer 3). The cost associated with edge Primer2/Primer 3 would be  $900 + 10 ([10])0 + 4000 = 4900$  (reflecting the 900 base overlap between the amplicons).